sizes, based on molecular weight, of the enzyme complexes, ultimately they appeal to "biochemical considerations" to defend this localization: "Complexes I and II must interact with DPNH and succinate, respectively, both of which are localized in the interior of the crista, whereas complex IV must interact with molecular oxygen which would be more readily available in the solution outside the crista rather than in its interior" (p. 95). Green presented a popularized account of this proposal in a paper in *Scientific American* in 1964. There he also offered a speculative proposal (attributed to Robert Bock and Robert Criddle), according to which the transport of substrates between enzymes was accomplished "by means of swinging groups of atoms, mounted on the respective proteins by flexible arms, that transfer and accept the electrons" (Green, 1964). This mechanism, it should be noted, addressed the issue of electron transport, but not the accompanying phosphorylation.

Green's proposals were quickly discounted by other biochemists. I noted previously that Racker had found that F_o preparations generated particles when salt was added. Collaborating with Donald Parsons and Britton Chance, he examined these preparations with the negative staining technique and found that they were sac-shaped structures covered with "inner membrane spheres" like those found by Fernández-Morán. He then treated the preparations with trypsin, followed by urea, a treatment he had previously employed to remove ATPase activity from his preparations. Examining these preparations with negative staining, he found they had no inner membrane spheres. This established that, contrary to Green's proposal, the electron transport chain was not in the spheres. On the other hand, preparations of the F_1 factor showed spheres about 85 Å in diameter, and when such preparations were added to the trypsin-urea membrane preparation, spheres appeared on the membrane. The preparation still did not perform oxidative phosphorylation, but when F_2, F_3, and F_4 were added as well, phosphorylation was restored (Racker et al., 1965; see also Racker, 1968). He concluded that the spheres contained the ATPase and were the locus of ATP synthesis.

Racker was not fully satisfied with the demonstration, however, because the difference in number of spheres before and after adding F_1 was small enough that he had to rely on statistical analysis to establish it. His research assistant, Lawrence Horstman, tried passing mitochondrial fractions through a Sephadex column in order to remove the native spheres more effectively.[24]

[24] Racker (1976, p. 16) commented, "He tried these experiments without any encouragement from me because I did not think that the procedure could be carried out without damage to the particles. However, it worked, which brings us to the next lesson, Lesson 6: Progress is made by young scientists who carry out experiments old scientists said wouldn't work (F. Westheimer)."